Supplemental Data

Perfluorinated Compounds in Surface Waters and Sediments in Northwest Georgia, USA, and Their Bioaccumulation in *Lumbriculus variegatus*

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Pages: 19

Tables: 14

Table S1: Locations of surface water and sediment collections.

Location	Latitude	Longitude
Hwy 411, TN	35° 00’ 60”N	84° 44’ 04”W
Brown Bridge Rd, GA	34° 42’ 52”N	84° 51’ 84”W
Tilton Bridge Rd, GA	34° 40’ 02”N	84° 55’ 72”W
Hwy 136, GA	34° 35’ 58”N	84° 56’ 04”W
Sugar Valley Rd, GA	34° 30’ 58”N	84° 57’ 45”W
Hwy 156, GA	34° 29’ 49”N	85° 00’ 86”W
Veterans Memorial Hwy, GA	34° 17’ 22”N	85° 09’ 97”W
Hwy 100, GA	34° 14’ 90”N	85° 21’ 34”W

Table S2: Water quality characteristics[1] of surface waters collected from eight sites along the Conasauga, Oostanaula and Coosa Rivers, Georgia.

Site	Conductivity (µS/cm)	Dissolved oxygen (% saturation)	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
1	38	95	8.1	30	36
2	185	93	7.9	100	104
3	196	88	7.9	100	96
4	201	83	7.9	96	100
5	125	94	8.1	62	56
6	142	82	7.9	64	60
7	149	88	7.9	70	72
8	227	86	7.9	78	84

[1]Conductivity - Orion™ model 1214000; pH - Orion™ model 720A with Triode electrode; dissolved oxygen - Orion™ model 1113000; alkalinity and hardness by titration (American Public Health Association, 1992).

Table S3: Physical characteristics[1] of sediment samples collected from eight sites along the Conasauga, Oostanaula and Coosa Rivers, Georgia.

Site	Replicate	Organic material (%)	Organic carbon (%)	Total nitrogen (%)	Sand (%)	Silt (%)	Clay (%)
1	1*	4.8	2.5	0.12	87.8	10.0	2.3
	2*	1.1	0.4	0.03	92.8	6.6	0.5
	3	3.9	1.3	0.07	90.8	7.2	2.0
2	1*	2.6	1.1	0.08	71.7	20.4	7.9
	2*	1.7	0.8	0.07	80.9	14.2	4.9
	3	4.5	1.7	0.14	42.4	45.6	12.0
3	1	4.4	1.7	0.12	47.7	39.6	12.7
	2*	2.7	1.0	0.08	77.2	16.2	6.6
	3*	1.5	0.4	0.04	83.5	12.1	4.4
4	1*	2.3	1.0	0.06	82.5	12.6	4.9
	2*	4.1	2.0	0.14	79.2	11.1	9.6
	3	6.2	2.4	0.22	20.7	60.8	18.6
5	1	3.8	1.4	0.08	66.9	23.7	9.4
	2*	3.7	1.4	0.10	74.4	18.4	7.1
	3*	4.1	1.8	0.11	58.8	31.5	9.7
6	1	1.9	0.7	0.06	86.4	11.4	2.2
	2*	6.6	2.5	0.21	41.7	44.8	13.5
	3*	6.0	2.6	0.22	31.4	55.2	13.4
7	1*	4.7	2.0	0.14	53.1	34.5	12.4
	2*	5.4	1.9	0.16	42.5	44.1	13.4
	3*	5.1	2.1	0.14	50.0	36.6	13.4
8	1*	1.6	0.5	0.04	71.2	20.0	8.8
	2*	1.8	0.5	0.04	66.4	23.9	9.6
	3*	3.7	1.4	0.10	68.3	24.3	7.4

[1] Organic material - Davies et al., 1974; organic carbon and total nitrogen - Leco CNS-2000 Analyzer; particle-size distribution - Miller and Miller, 1987.

* collected from the same side of the river.

Table S4: Metal concentrations (mg/kg dw)[1] in sediments collected from eight sites along the Conasauga, Oostanaula and Coosa Rivers, Georgia.

Site	Replicate	Na	Mg	K	Ca	Mn	Fe	FeOH	Cr	Cu	Ni	Pb	Zn
1	1	2885	1909	1003	4722	275	16268	96	26	11	11	12	51
	2	2939	1698	930	2521	86	11342	24	23	5	7	6	32
	3	3420	2309	1105	4070	267	15870	134	27	8	11	13	45
2	1	3579	2893	968	4506	1044	19682	87	34	13	14	18	50
	2	2658	3811	1036	6677	1482	21170	107	35	11	16	16	39
	3	3558	3690	1196	4777	1288	22536	154	46	17	21	20	74
3	1	2982	3885	1255	3918	1647	26097	202	54	18	22	28	143
	2	2680	2224	898	3712	810	13128	45	24	9	10	12	38
	3	1869	1479	886	2573	402	8370	71	16	4	7	8	15
4	1	2465	2382	934	4654	817	13997	66	25	9	11	12	48
	2	2393	2279	1055	3908	694	32042	122	35	13	18	21	82
	3	3573	5966	1736	5797	2370	31178	217	57	22	28	26	123
5	1	2865	3666	1340	3926	1012	22128	186	34	21	16	16	73
	2	3046	3729	1373	4817	947	22404	90	35	15	15	19	59
	3	2989	3864	1387	3633	1126	22160	141	35	15	16	17	61
6	1	2002	2099	1001	3284	559	14797	78	23	9	9	12	32
	2	3391	5250	1839	5054	1598	31434	215	51	23	25	30	112
	3	3082	4493	1558	4892	1301	25826	503	43	20	21	26	90
7	1	2758	3941	1501	4499	1094	24399	212	41	17	19	24	88
	2	2537	3938	1452	4339	1208	23396	147	39	16	18	23	81
	3	2988	4527	1626	4762	1254	25954	435	43	18	20	26	95
8	1	6326	4050	1412	6761	773	23161	116	37	13	15	25	53
	2	4956	3382	1129	5258	633	17180	126	35	22	22	15	52
	3	5476	3853	1271	6591	690	25215	137	34	18	17	27	85

[1] Metals - USEPA; FeOH - Jenne and Crecelius, 1988.

Chemicals.
Except as noted below, all chemicals used in this study were of the highest purity offered by the suppliers, uniformly ≥97% purity. Perfluoro-n-hexanoic acid, perfluoro-n-octanoic acid, perfluoro-n-nonanoic acid, perfluoro-n-decanoic acid, perfluoro-n-[1,2-^{13}C]hexanoic acid, perfluoro-n-[1,2,3,4-^{13}C]octanoic acid, perfluoro-n-[1,2,3,4,5-^{13}C]nonanoic acid, perfluoro-n-[1,2-^{13}C]decanoic acid all were purchased as certified standards from Wellington Laboratories through TerraChem (Shawnee Mission, KS, USA). Formulae for these perfluorocarboxylic acids (PFCAs) and the acronyms used herein for these compounds, are summarized in Table S5. Tetrabutylammonium hydrogen sulfate (TBAHS) and sodium carbonate, were purchased from Aldrich Chemical (Milwaukee, WI, USA). Acetonitrile (ACN), glacial acetic acid, methanol (MeOH) and methyl *tert*-butyl ether (MTBE) were purchased from Fisher Chemical (Fairlawn, NJ, USA). Oasis HLB solid-phase extraction (SPE) cartridges, 35-cm^3 capacity, were purchased from Waters (Milford, MA, USA).

Sampling preparation, methods, detection limits, and QA/QC for analyses of perfluorinated chemicals.
Prior to field collections, all sampling utensils and sample containers were washed in soap and water, rinsed thoroughly with deionized water and allowed to dry and then rinsed three separate times with Optima-grade methanol. Once cleaned, sampling utensils were wrapped in plastic wrap (Saran Wrap®) for transport to the field. Field blanks were collected at each site for sediment and water samples. A sample of clean sand was transported to the field in a 250-mL HDPE bottle, poured over all utensils used to collect and homogenize sediments at that site and then returned to its bottle for processing identical to the collected sediment samples. Blank water samples consisted of an aliquot of polished water transported to the field, exposed to air during sample collection and returned to the laboratory for analyses.

Preparation of Water Samples for Analysis. A 9.88 ml aliquot of water sample was transferred to an HDPE vial. This aliquot was spiked with ~0.138 g of 96%/4% acetonitrile/water containing mass-labeled matrix internal standards at 6.1 ng/g. This treatment yielded samples consisting of about 99% water and 1% acetonitrile, by mass, containing 84 pg/g of matrix internal standards, the same concentration that the calibration standards contain. Mass-labeled matrix internal standards included (M+4)perfluorobutanoic acid, (M+2)perfluorohexanoic acid, (M+4)PFOA, (M+5)perfluorononanoic acid, (M+2)perfluorodecanoic acid (PFDA or C10), (M+2)perfluoroundecanoic acid, (M+2)perfluorododecanoic acid, (M+2)6:2-fluorotelomer unsaturated carboxylic acid (FTUCA), (M+2)8:2-FTUCA, and (M+2)10:2-FTUCA. Spiked samples were transferred to polypropylene autosampler vials. All samples were analyzed by ultra-performance liquid chromatography, tandem mass spectrometry operated in negative electrospray-ionization mode as described below. Analytes included perfluorocarboxylic acids C4 through C14, FTUCAs 6:2, 8:2 and 10:2 (not reported here), and

perfluorosulfonates C4 (PFBS), C6, C7 and C8 (PFOS). Water samples were collected from the laboratory tap for comparison to the sampled systems. Deionized (18 MΩ) water was polished by elution through an SPE cartridge to represent zero concentration of the analytes.

Extraction of Sediment Samples. For the sediment samples, 1-g aliquots were transferred from each of three replicate field samples collected at each of the eight sampling locations to 16-mL polypropylene copolymer (PPCO) centrifuge tubes that were sealed with PPCO press-on caps. For these sediment samples, we used a modification of an ACN/H_2O extraction we reported upon earlier (Washington et al., 2008) by extracting each sample four times with 60/40 ACN/H_2O and mixing during extraction on a Labquake rotisserie, as opposed to a shaker table, but otherwise following our published method. Although we modified our extractions as described above to accommodate their PFA-contaminated nature, we retained all other practices from our published methods (Washington et al, 2008) including: i) spiking samples prior to extraction with $^{13}C_8$-PFOA as a recovery internal standard; ii) subjecting extracts to ion-pairing cleanup to decrease analytical noise from natural organic matter that normally is concentrated in surface soils; iii) reconstituting extracts in 60/40 ACN/H2O with a suite of mass-labeled PFAs present (Table SI5) at 84 pg/g as matrix internal standards; and iv) running procedural blanks in which the extraction process was carried out on otherwise-empty extraction tubes.

Extraction of Oligochaetes. Oligochaete samples, consisting of a composite of approximately 100 to 350 individuals (0.9-3.6 g ww), were frozen in pre-weighed 16-mL polypropylene copolymer (PPCO) centrifuge tubes that were sealed with a PPCO press-on caps until extraction. Composites weighing more than 2 g were split into separate samples for analysis. These samples were extracted using a method modified from Henderson et al. (2007) by adding 0.5-1 mL of 18 MΩ H_2O to the thawed sample then vortexing to homogenize. To this homogenate, 100 µL of 10 ng/g $^{13}C_8$-PFOA was added as a recovery internal standard, vortexed and reweighed. Then 4.0 mL of 0.25 M tetrabutylammonium hydrogen sulfate was added followed by the addition of 5.0 mL of methyl tert-butyl ether (MTBE). Tubes then were placed on a Labquake rotisserie, rotated for 15 to 24 hrs, centrifuged at 650 G and 18 to 22 ^{o}C for 5 min., the aqueous phase frozen and the MTBE transferred to a capped, preweighed 12-mL vial that was stored in the freezer. The sample was rethawed, 3 mL of MTBE added, rotisseried for 15 to 24 hr, centrifuged, frozen and the MTBE transferred to the previously dosed vial which was returned to the freezer. The sample then was extracted a third time with 3 mL of MTBE identically to the second extraction and the third MTBE extraction was added to the previous two. The combined MTBE extracts were evaporated to dryness in an SPE assembly, using nylon syringe filters to filter the air, under a 5-psi vacuum. The samples then were reconstituted in 60:40 ACN:H_2O containing a suite of mass-labeled PFAs present (Table SI5) at 84 pg/g as matrix internal standards.

Liquid Chromatograph, Tandem Mass-Spectrometer Analyses. Acetonitrile/water extracts were analyzed on a Waters Acquity ultra-performance liquid chromatograph (UPLC) interfaced with a Waters Quattro Premier XE tandem mass spectrometer operated in negative electrospray-ionization mode. We integrated peaks by setting software integration parameters so that integrations closely approximated the integration rules described in detail in Washington et al. (2007). All software-integrated peaks were checked and, if needed, adjusted manually according to these same integration rules. Efforts were made to reduce background noise in the system for PFOA by modifying the UPLC plumbing. Modifications included installation of polyaryletheretherketone (PEEK) tubing, removal of the degasser, installation of a C18 trap column (100mm × 2.1mm × 3.5µm) in the water eluent line immediately upgradient of the solvent mixer, and use of manually-degassed 18 MΩ water "polished" by passing through a Waters HLB solid-phase extraction cartridge (Washington et al., 2008).

All system operations were controlled by Waters MassLynx 4.1 and QuanLynx 4.1. Twenty microliters of extract were introduced to a Waters BEH C18 guard cartridge followed by a Waters BEH C18 analytical column, 100mm × 2.1mm × 2.1µm, maintained at 35 ^{o}C. The UPLC was operated using ACN and water eluents adjusted to pH 4 with glacial acetic acid. Pumping at a constant total flow of 0.5 mL/min, runs were started with 35% ACN, and then linearly ramped to 90% ACN over 5 min, held for 6 min, linearly ramped back to 35% ACN at 11.1 min, from which time the composition was held constant until the end of analysis at 13 min.

After UPLC elution, extracts were introduced to the mass spectrometer operated in negative electrospray ionization (ESI(-)) mode with the capillary potential set at -600 V, the extractor potential at -2 V and the radio-frequency (RF) lens potential at 0.3 V. The source temperature was maintained at 140 ^{o}C. The N_2 generator desolvation gas was maintained at 350 ^{o}C and 800 L/h flow. The cone gas flow, also supplied by the N_2 generator, was set to 25 L/h. Analyte-specific instrumental parameters, including monitored transitions, were optimized for PFCs analysis (Table S5). The low- and high-mass resolutions in the first quadrupole both were set to 13.0 (unitless ratio of direct to RF current voltages) and the ion energy was set to 0.7 eV. In the collision cell, a traveling-wave ion guide (TWIG), the entrance was set to -3 V, the interior set to -16 V and the exit set to -1 V. The Ar collision gas was set to flow at 0.45 mL/m. Low- and high-mass resolutions in the second quadrupole both were set to 12.0 and the ion energy was set to 1.0 eV. The detector was operated in multiple-reaction-monitoring (MRM) mode, with the detector multiplier set to -700 V and the dwell time was set to 70 ms with the objective of achieving at least 15 scans per peak.

Table S5: Liquid Chromatographic and Mass Spectrometric Settings

Compound	Nominal Retention Time: Apex RT (min)	Front RT (min)	Tail RT (min)	Delta T from prev Apex (min)	Number of transitions	Number of transitions per function	Parent Anion mass (m/z)	Cone potential (V)	Quan Ion mass (m/z)	Quan Ion collision energy (eV)	Primary Qual Ion mass (m/z)	Primary Qual Ion collision energy (eV)	2nd Qual Ion mass (m/z)	2nd Qual Ion collision energy (eV)
Function 1 Time Interval 0 to 1.1 Min														
Perfluoroprionoic acid (C3)	0.65	0.4	0.9		2	5	162.80	14	118.80	11	69.80	25		
Perfluorobutanoic acid (C4; PFBA)	0.70	0.4	1.0	0.05	1		212.85	13	168.80	10	Irregular response			
$^{13}C_4$-Perfluorobutanoic acid ((M+4)C4;MPFBA	0.70	0.4	1.0	0.05	1		216.90	14	171.80	10	Irregular response			
Perfluoro pentanoic acid (C5;PFPA)	0.95	0.6	1.3	0.25	1		262.80	13	218.85	10	Irregular response			
Function 2 Time Interval 0.9 to 2.1 Min														
Perfluorohexanoic acid (C6; PFHxA)	1.35	1.0	1.7	0.40	2	7	312.80	13	268.85	10	118.80	20		
$^{13}C_2$-Perfluorohexanoic acid ((M+2)C6; MPFHxA)	1.35	1.0	1.7	0.40	1		314.80	14	269.85	10	119.30	20		
Perfluorobutane sulfonate (S4; PFBS)	1.50	1.2	1.8	0.15	2		298.90	40	79.85	30	98.85	40		
Perfluoroheptanoic acid (C7; PFHpA)	1.80	1.5	2.1	0.30	2		362.70	13	318.80	10	168.85	18		
Function 3 Time Interval 1.8 to 3.2 Min														
Perfluorooctanoic acid (C8; PFOA)	2.30	1.9	2.7	0.50	2	11	412.70	14	368.75	10	168.85	18		
$^{13}C_4$-Perfluorooctanoic acid ((M+4)C8; M4PFOA	2.30	1.9	2.7	0.50	1		416.70	14	371.70	10	171.85	18		
$^{13}C_8$-Perfluorooctanoic acid ((M+8)C8; M8PFOA	2.30	1.9	2.7	0.50	1		420.70	13	375.70	11	171.85	20		
Perfluorohexane sulfonate (S6; PFHxS)	2.50	2.1	2.9	0.20	2		398.90	50	79.85	40	98.85	40		
Perfluorononanoic acid (C9; PFNA)	2.75	2.4	3.1	0.15	2		462.70	15	418.70	11	218.85	18		
$^{13}C_5$-Perfluorononanoic acid ((M+5)C9; MPFNA	2.75	2.4	3.1	0.15	1		467.70	15	422.70	12	222.90	18	218.9	18
Function 4 Time Interval 2.4 to 3.4 Min														
Perfluoroheptane sulfonate (S7; PFHpS)	2.95	2.6	3.3	0.20	2	2	448.90	50	79.90	40	98.90	40		
Function 5 Time Interval 2.9 to 4.4 Min														
Perfluorodecanoic acid (C10; PFDA)	3.35	3.0	3.7	0.40	2	11	512.90	15	468.70	11	218.85	20		
$^{13}C_2$-Perfluorodecanoic acid ((M+2)C10; MPFDA)	3.35	3.0	3.7	0.40	1		514.90	15	470.00	12				
Perfluorooctane sulfonate (S8; PFOS)	3.55	3.2	3.9	0.20	2		498.90	60	79.85	50	98.85	40		
8:2 Fluorotelomer unsaturated acid (8:2FTUCA)	3.65	3.3	4.0	0.10	2		456.70	16	392.70	18	342.70	40		
$^{13}C_2$- 8:2 Fluorotelomer unsaturated acid ((M+2)8:2FTUCA)	3.65	3.3	4.0	0.00	1		458.70	16	393.70	16	343.70	40		
Perfluoroundecanoic acid (C11; PFUnDA)	3.90	3.6	4.2	0.25	2		562.70	15	518.70	12	218.85	20		
$^{13}C_2$-Perfluoroundecanoic acid ((M+2)C11; MPFUnDA)	3.90	3.6	4.2	0.25	1		564.90	15	520.00	13				
Function 6 Time Interval 4.0 to 15.0 Min														
Perfluorododecanoic acid (C12 PFDoDA)	4.50	4.2	4.8	0.60	2	10	612.70	16	568.70	13	318.70	20		
$^{13}C_2$-Perfluoro dodecanoic acid ((M+2)C12; MPFDoDA)	4.50	4.2	4.8	0.60	1		614.90	16	570.00	13				
10:2 Fluorotelomer unsaturated acid (10:2FTUCA)	4.65	4.4	4.9	0.15	2		557.00	16	493.00	17	443.00	38		
$^{13}C_2$-10:2 Fluorotelomer unsaturated acid ((M+2)10:2FTUCA)	4.65	4.4	4.9	0.15	1		559.00	16	494.00	17				
Perfluorotridecanoic acid (C13; PFTrDA)	5.15	4.9	5.4	0.50	2		662.75	16	618.70	13	318.70	22		
Perfluorotetradecanoic acid (C14; PFTeDA)	5.80	5.5	6.1	0.65	2		712.75	18	668.70	14	318.70	24		

Raw Data Optimization & Quantitation: Chromatograms were smoothed using a second-order Savitsky-Golay algorithm and two five-point smoothes with a few exceptions to accommodate monitoring the high number of transitions in the method (Table S6). Quantitation was performed using mass-labeled matrix internal standards. Quantitation for C4, C6, C8, C9, C10, C11, and C12, analytes was accomplished using isotopic dilution since isotopically labeled standards were available. C5 was quantitated using the mass-labeled C6 ($^{13}C_2$-PFHxA), and C7 and PFOS were quantitated using the mass-labeled C8 ($^{13}C_4$-PFOA) and C10 ($^{13}C_2$-PFDA) matrix internal standards, respectively. Calibrations were constructed with linear regressions of untransformed data, and plots of peak area/internal standard area versus calibration standard concentration/ internal standard area; 1/X weighting was applied for regression. Standards injected on the instrument ranged from 0.9 to 4800 pg/g. The lowest standard concentrations that were used to generate the calibration curves were those levels for which the calibration lines maintained a central tendency for repeated measures of the standards. Final calibration curves consisted of 11-14 standard concentrations of the targeted species spanning from 5 to 4800 pg/g. Standards were interspersed with sample extracts and blanks throughout the sample-analysis runs. Sample extracts were diluted as needed to get their concentrations to fall within the instrument calibration range using 60:40 ACN:H_2O spiked with appropriate concentrations of all matrix internal standards.

Table S6: Typical Integration and Optimization Parameters

Compound	Savitzky Golay Smoothing # points; # smooths	Quantitative Qualitative ratio & tolerance (%)	Internal standard
Function 1 Time Interval 0 to 1.1 Min			
Perfluoroprionoic acid (C3)	5; 2		
Perfluorobutanoic acid (C4; PFBA)	5; 2		
$^{13}C_4$-Perfluorobutanoic acid ((M+4)C4;MPFBA	5; 2		
Perfluoro pentanoic acid (C5;PFPA)	0; 0		
Function 2 Time Interval 0.9 to 2.1 Min			
Perfluorohexanoic acid (C6; PFHxA)	5; 2	21.0 ± 44%	(M+2)C6
$^{13}C_2$-Perfluorohexanoic acid ((M+2)C6; MPFHxA)	5; 2		
Perfluorobutane sulfonate (S4; PFBS)	5; 2	4.8± 44%	(M+2)C6
Perfluoroheptanoic acid (C7; PFHpA)	0; 0	3.1± 44%	(M+4)C8
Function 3 Time Interval 1.8 to 3.2 Min			
Perfluorooctanoic acid (C8; PFOA)	5; 2	3.31± 44%	(M+4)C8
$^{13}C_4$-Perfluorooctanoic acid ((M+4)C8; M4PFOA	5; 2		
$^{13}C_8$-Perfluorooctanoic acid ((M+8)C8; M8PFOA	5; 2		(M+4)C8
Perfluorohexane sulfonate (S6; PFHxS)	5; 2	2.0± 44%	(M+4)C8
Perfluorononanoic acid (C9; PFNA)	5; 2	4.3± 44%	(M+5)C9
$^{13}C_5$-Perfluorononanoic acid ((M+5)C9; MPFNA	5; 2		
Function 4 Time Interval 2.4 to 3.4 Min			
Perfluoroheptane sulfonate (S7; PFHpS)	5; 2	1.5± 44%	(M+5)C9
Function 5 Time Interval 2.9 to 4.4 Min			
Perfluorodecanoic acid (C10; PFDA)	5; 2	6.8± 44%	(M+2)C10
$^{13}C_2$-Perfluorodecanoic acid ((M+2)C10; MPFDA)	5; 2		
Perfluorooctane sulfonate (S8; PFOS)	5; 2	1.32± 44%	(M+2)C10
8:2 Fluorotelomer unsaturated acid (8:2FTUCA)	5; 2		(M+2)8:2FTUCA
$^{13}C_2$- 8:2 Fluorotelomer unsaturated acid ((M+2)8:2FTUCA)	5; 2		
Perfluoroundecanoic acid (C11; PFUnDA)	5; 2	8.8± 44%	(M+2)C11
$^{13}C_2$-Perfluoroundecanoic acid ((M+2)C11; MPFUnDA)	5; 2		
Function 6 Time Interval 4.0 to 15.0 Min			
Perfluorododecanoic acid (C12 PFDoDA)	5; 2	10.8± 44%	(M+2)C12
$^{13}C_2$-Perfluoro dodecanoic acid ((M+2)C12; MPFDoDA)	5; 2		
10:2 Fluorotelomer unsaturated acid (10:2FTUCA)	5; 2		(M+2)10:2FTUCA
$^{13}C_2$-10:2 Fluorotelomer unsaturated acid ((M+2)10:2FTUCA)	5; 2		
Perfluorotridecanoic acid (C13; PFTrDA)	5; 2	12.9± 44%	(M+2)C12
Perfluorotetradecanoic acid (C14; PFTeDA)	5; 2	16.9± 44%	(M+2)C12

Method detection limits (MDL) and limits of quantitation (LOQ). MDL and LOQ were calculated using four repeated measures of the 18 pg/g standard. MDL was calculated with Students t test by t times standard deviation of the four repeated measures of the 18 pg/g standard where $t_{0.01} = 4.451$ for n=4. LOQ was defined as 10 times the standard deviation.

Table S7: Method detection limits (MDL) and limits of quantitation (LoQ)

	Surface water (ng/g)		Sediment (ng/g dw)		Tissue (ng/g ww)	
Homologue	MDL	LoQ	MDL	LoQ	MDL	LoQ
PFBA	0.012	0.028				
PFPA	0.017	0.038				
PFHxA	0.011	0.024	0.131	0.349	0.174	0.383
PFHpA	0.009	0.019	0.036	0.095	0.105	0.232
PFOA	0.007	0.016	0.036	0.095	0.023	0.051
PFNA	0.005	0.010	0.032	0.084	0.133	0.294
PFDA	0.011	0.023	0.023	0.060	0.066	0.146
PFUnDA			0.121	0.322	0.132	0.291
PFDoDA			0.126	0.337	0.096	0.211
PFTrDA			0.082	0.220	0.124	0.274
PFTeDA			0.039	0.104	0.083	0.182
PFBS	0.020	0.044	0.054	0.144	0.077	0.169
PFHxS	0.014	0.031	0.074	0.197	0.102	0.225
PFHpS	0.028	0.061	0.297	0.794	0.097	0.214
PFOS	0.009	0.019	0.149	0.398	0.160	0.353

Results.

Method detection limits could not be calculated for PFUnDA, PFDoDA, PFTrDA, and PFTeDA in surface-water samples due to the lack of any observable peak for these homologues; safe to say MDL was at least as low as the most insensitive PFCA, C3 MDL = 0.028 ng/g; C3 LoQ = 0.062 ng/g. Method detection limits could not be calculated for PFBA and PFPA in sediment and tissue due to excessive noise in the extracts. Blank samples of water, sediment, and tissues were below the mdl for all homologues. Mean recoveries of the M8C8 internal reference were 83% (CV = 8) and 92% (CV = 12) for the sediment and tissue analyses, respectively.

Within-site variability in PFC concentrations was greatest in tissue samples (mean CV = 86%, for 11 homologues), followed by sediment samples (mean CV = 46%, for 11 homologues), and lowest among water samples (mean CV = 10% for 7 homologues). Variability among analyte concentrations in split oligochaete samples was low. Mean CVs for samples exceeding analyte MDL were as follows: PFHxA 31%, n=5; PFHpA 25%, n = 10, PFOA 18%, n = 11; PFNA 11%, n = 9; PFDA 5%, n = 11; PFUnDA 4%, n = 11; PFDoDA 3%, n = 11; PFTrDA 4%, n = 11; PFTeDA 4%, n = 11; PFBS 26%, n = 11; PFHxS 26%, n = 11; PFHpS 18%, n = 9; PFOS 6%, n = 11.

Table S8. Mean concentrations (ng/L) of perfluorinated chemicals (with coefficient of variation, *n* = 3) in surface waters collected from sites along the Conasauga, Oostanaula and Coosa Rivers.

	Site							
Homologue	1	2	3	4	5	6	7	8
PFBA	< MDL	< MDL	53* (9)	81* (12)	26* (12)	27* (6)	33* (21)	34* (2)
PFPA	< MDL	< MDL	125* (15)	149* (18)	57* (15)	61* (12)	56* (14)	65* (2)
PFHxA	< MDL	< MDL	112* (6)	149* (16)	53* (8)	72* (5)	64* (10)	94* (14)
PFHpA	< MDL	< MDL	89* (16)	100* (14)	43* (4)	51* (15)	48* (8)	38* (8)
PFOA	< MDL	13 (28)	193* (10)	204* (13)	100* (2)	134* (14)	113* (10)	104* (9)
PFNA	< MDL	< MDL	35* (5)	44* (14)	17* (3)	21* (8)	20* (16)	21* (22)
PFDA	< MDL	< MDL	45* (20)	46* (19)	21* (16)	29* (5)	28* (20)	20* (28)
PFBS	< MDL	< MDL	205* (11)	260* (7)	125* (1)	134* (3)	122* (10)	105* (12)
PFHxS	< MDL	< MDL	30* (4)	31* (7)	17* (7)	13* (45)	< MDL	<MDL
PFHpS	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
PFOS	< MDL	< MDL	297* (6)	321* (9)	152* (10)	148* (14)	151* (28)	83* (22)

*Concentration is significantly greater than those measured at sites 1 and 2 (Dunnett's one-tailed test, $\alpha \leq 0.05$).

Stability of Mixed Perfluoroalkylates in Water.

A mixture containing 5000 ng each of the perfluoroalkylates (PFCAs) C4 – C14 and the perfluorosulfonates (PFSAs) S4 and S6-S8, was prepared from 650 ng/g stock solutions in 60/40 Acetonitrile/Polished 18 MΩ Water (ACN/H_2O). The mixture was prepared in a 250 mL Nalgene wide mouth bottle, rinsed 3X with HPLC grade methanol prior to use. The resulting mixture was subjected to house vacuum overnight and then diluted to 250 mL with polished 18 MΩ Water. The intent of placing this mixture under vacuum was to

accelerate preferential evaporation of ACN from the ACN/H_2O solution, a phenomenon we had observed in past practice with ACN/H_2O solutions. Residual ACN content was not determined. The average PFAA concentration in this stock mixture was 18 ng/g.

Two 125 mL aqueous solutions were prepared from this stock mixture, both diluted to an average theoretical concentration of 93 pg/g with polished 18 MΩ Water. These final aqueous test solutions contained less than 0.14% ACN on a mass basis using the highly conservative assumption that no ACN was lost during the earlier evaporation step. One of these solutions was kept at room temperature, about 22 ^{o}C; the other was refrigerated at 7.5 °C. All solution transfers were made with an Eppendorf pipetter; the pipette tip was submerged at least one-half inch below the surface for aliquot removal. All dilution quantitations are based upon gravimetric measurements.

For LC/MS/MS analysis, 400 µL of each solution was mixed with 600 µL of a mixture of mass-labeled quantitation standards in 60/40 ACN/polished 18 MΩ water. The concentration of each standard in the mass-labeled quantitation standards stock solution was 177 pg/g, giving a concentration in the autosampler vial of about 95 pg/g. The resulting concentration of ACN in the autosampler vial is sufficient to prevent adsorption of analytes to the glass vial. One sample vial was prepared from the room-temperature solution and one from the refrigerated solution per sampling event; only for T_0 were both samples at collected from room-temperature solutions. Sampling events were staged daily for eight days, including T_0 = 30 minutes after mixing the 93 pg/g solutions. Each sample vial was subjected to three repeated measures on the LC/MS/MS.

One day and later after making the aqueous test solutions, no analytes varied with time with statistical significance, except for the room temperature PFBA which trended downward from day 1 through day 7. Mean recoveries and standard deviations for day 1 through day 7 data are tabulated in Table 1. Each mean in Table 1 represents the mean of seven samples, day 1 through day 7, with each sample represented by three repeated measures for a total of 21 data points for each mean. Non-detects for C13 and C14 were entered as zeros for statistical purposes.

Table S9: Recoveries from water-stability study.

	Percent recovery (n = 21)			
Homologue	Refrigerated (7.5 °C)		Room temperature	
	Mean (n = 3)	Standard deviation	Mean (n = 3)	Standard deviation
PFBA	114	11	97	16
PFPA	87	7	82	4
PFHxA	96	6	90	5
PFHpA	82	4	80	7
PFOA	87	5	83	6
PFNA	82	10	80	8
PFDA	80	7	78	9
PFUnDA	69	6	62	3
PFDoDA	26	5	20	3
PFTrDA	3	4	3	1
PFTeDA	0	2	0	1
PFBS	93	5	88	5
PFHxS	90	5	88	4
PFHpS	95	17	94	20
PFOS	66	8	65	5

There was a slight and consistent, increase in recovery for refrigerated storage vs. room temperature storage when the compounds were evaluated individually; this small difference does not seem to be attributable to differences in density based on calculations of density for pure water at 7.5 and 22 ^{o}C (representing room temperature). Only C4 at 7.5 ^{o}C was recovered at 100% and time-invariant; as noted above, room temperature C4 had a statistically significant downward trend over day 1 to day 7. Recoveries for C5-C10 are in the 78% - 96% range, as are the three lower molecular weight PFSAs. Beginning with C11 and S8, recovery drops to less than 70%, while C12 drops to 20 to 26%, and C13 and C14 recovery approaches zero.

Table S10. Mean concentrations (ng/g, dw) of perfluorinated chemicals (with coefficient of variation, $n = 3$) in sediments collected from sites along the Conasauga, Oostanaula and Coosa Rivers.

	Site							
Homologue	1	2	3	4	5	6	7	8
PFHxA	< MDL	< MDL	0.15 (100)	0.40 (128)	< MDL	< MDL	< MDL	< MDL
PFHpA	< MDL	< MDL	0.16 (70)	0.39[b] (100)	0.07 (18)	0.08 (18)	0.09 (27)	0.04 (101)
PFOA	0.06 (82)	0.15 (43)	0.74 (59)	1.97[b] (104)	0.33 (26)	0.47 (12)	0.45 (20)	0.26 (95)
PFNA	0.03 (62)	0.08 (76)	0.25 (50)	0.68[b] (69)	0.14 (53)	0.21 (12)	0.27 (8)	0.07 (75)
PFDA	0.03 (100)	0.50 (46)	2.12[a] (56)	4.66[b] (42)	1.51 (55)	2.09[a] (22)	2.67[b] (12)	0.35 (82)
PFUnDA	< MDL	0.36 (26)	2.63[b] (38)	3.80[b] (58)	2.50[a] (53)	3.53[b] (26)	3.53[b] (17)	0.33 (87)
PFDoDA	< MDL	1.00 (22)	4.64[b] (84)	4.60[b] (48)	2.52 (35)	2.97 (12)	3.00 (17)	0.98 (138)
PFTrDA	0.07 (75)	0.30 (22)	0.98[a] (59)	0.99[a] (65)	0.59 (31)	0.80[a] (10)	0.93[a] (19)	0.21 (139)
PFTeDA	0.05 (102)	0.52 (26)	1.67[a] (87)	1.19 (58)	0.66 (25)	0.68 (13)	0.70 (20)	0.30 (148)
PFBS	0.05 (87)	< MDL	.11 (73)	0.17[b] (16)	0.09 (14)	0.22[b] (38)	0.20[b] (15)	0.10 (16)
PFHxS	< MDL	< MDL	0.07 (83)	0.17[b] (92)	< MDL	< MDL	< MDL	< MDL
PFHpS	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
PFOS	< MDL	1.73 (39)	7.24 (67)	20.18[b] (54)	5.10 (42)	6.05 (24)	8.70 (14)	1.66 (103)

[a] Concentration is significantly greater than measured at site 1; [b] Concentration is significantly greater than measured at sites 1 and 2 (Dunnett's one-tailed test, $\alpha \leq 0.05$). MDL = method detection limit.

Table S11: Pearson correlation coefficients (r) with alpha values (n = 18) for relationships between concentrations of PFC homologues in sediment and sediment concentrations of organic carbon, total iron, amorphous iron, or clay.

Homologue	Organic C	Total Fe	FeOH	Clay
PFHpA	0.25	0.40	-0.07	0.10
	0.3094	0.0975	0.7853	0.7007
PFOA	0.26	0.42	-0.06	0.10
	0.2977	0.0862	0.8077	0.6932
PFNA	0.46	0.53	0.06	0.35
	0.0562	0.0251	0.8058	0.1550
PFDA	0.66	0.58	0.23	0.57
	0.0030	0.0124	0.3552	0.0133
PFUnDA	0.74	0.42	0.37	0.62
	0.0005	0.0862	0.1353	0.0066
PFDoDA	0.48	0.35	0.17	0.47
	0.0455	0.1488	0.5008	0.0473
PFTrDA	0.60	0.35	0.32	0.55
	0.0082	0.1508	0.2016	0.0178
PFTeDA	0.33	0.27	0.10	0.39
	0.1807	0.2784	0.6825	0.1095
PFBS	0.39	0.31	0.28	0.21
	0.1072	0.2079	0.2656	0.4130
PFOS	0.52	0.66	0.11	0.41
	0.0253	0.0148	0.6581	0.0885

Table S12: Mean concentrations (ng/g,dw) of perfluorinated chemicals (with coefficients of variation, $n = 3$) in sediments collected from site 3 in November, 2006 and in June, 2008.

Homologue	2006	2008
PFHxA	1.12 (18)	0.15 (100)*
PFHpA	0.81 (12)	0.16 (70)
PFOA	4.56 (19)	0.74 (59)
PFNA	2.32 (26)	0.25 (50)
PFDA	10.15 (24)	2.12 (56)

*Mean includes samples with concentrations below the method detection limit.

Table S13. Mean concentrations (ng/g, ww) of perfluorinated chemicals (with coefficient of variation, $n = 3$) in tissues of *Lumbriculus variegatus* exposed to sediments collected from sites along the Conasauga, Oostanaula and Coosa Rivers.

	Site							
Homologue	1	2	3	4	5	6	7	8
PFHxA	< MDL	< MDL	0.37 (14)	1.31 (125)	0.26 (34)	0.19 (74)	0.30 (34)	0.29 (63)
PFHpA	0.16 (59)	0.30 (19)	0.52 (31)	4.29[b] (120)	0.75 (27)	1.58 (26)	0.95 (55)	0.32 (74)
PFOA	0.10 (69)	0.60 (67)	2.06 (11)	11.80 (136)	1.35 (39)	2.11 (13)	2.19 (30)	0.95 (13)
PFNA	< MDL	0.82 (103)	2.28 (7)	11.04[b] (108)	1.68 (44)	2.61 (12)	4.29 (24)	0.90 (15)
PFDA	0.35 (82)	6.36 (40)	24.71 (14)	76.58[b] (58)	26.28 (36)	35.28[a] (16)	50.44[b] (7)	6.10 (27)
PFUnDA	1.13 (85)	5.79 (12)	34.87[b] (25)	58.09[b] (17)	48.65[b] (26)	60.34[b] (10)	69.42[b] (2)	11.50 (42)
PFDoDA	2.00 (80)	18.47 (9)	56.77[b] (14)	87.02[b] (7)	63.62[b] (27)	65.48[b] (15)	67.92[b] (1)	27.78 (100)
PFTrDA	2.90 (67)	8.90 (32)	21.52[b] (4)	34.15[b] (14)	24.10[b] (28)	33.04[b] (12)	35.81[b] (1)	10.98 (92)
PFTeDA	1.55 (81)	15.74 (40)	33.86[b] (32)	46.06[b] (9)	28.49[a] (29)	30.40[a] (7)	28.34[a] (7)	13.30 (122)
PFBS	1.91 (37)	1.66 (28)	3.30[b] (15)	3.52[b] (25)	2.61 (11)	3.03[a] (22)	2.63 (22)	2.36 (17)
PFHxS	5.01 (63)	4.16 (44)	2.89 (33)	12.21[c] (43)	9.72 (21)	13.87[b] (43)	8.30 (50)	5.34 (69)
PFHpS	0.15 (117)	0.29 (145)	2.39 (8)	19.31[b] (132)	1.95 (35)	2.66 (8)	3.30 (38)	1.04 (130)
PFOS	1.61 (91)	39.15 (56)	154.18 (28)	640.55[b] (79)	179.89 (33)	212.54 (19)	297.29 (15)	57.36 (73)

[a] Concentration is significantly greater than measured at site 1; [b] Concentration is significantly greater than measured at sites 1 and 2; [c] Concentration is significantly greater than measured at site 2 (Dunnett's one-tailed test, $\alpha \leq 0.05$).

Table S14. Pearson correlation coefficients (r) with alpha values (n = 18) for relationships between concentrations of PFCs in oligochaete tissue and sediment concentrations of PFCs (unmodified or normalized to sediment concentrations of organic carbon, total iron, iron oxides, or clay).

		Normalization			
Homologue	Unmodified	Organic C	Total Fe	Fe oxides	Clay
PFHpA	0.93	0.78	0.82	0.87	0.80
	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
PFOA	0.97	0.85	0.89	0.93	0.83
	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PFNA	0.93	0.74	0.81	0.88	0.74
	<0.0001	0.0005	<0.0001	<0.0001	0.0005
PFDA	0.86	0.59	0.69	0.65	0.53
	<0.0001	0.0107	0.0014	0.0033	0.0252
PFUnDA	0.80	0.43	0.67	0.38	0.39
	<0.0001	0.0721	0.0022	0.1190	0.1126
PFDoDA	0.60	0.47	0.58	0.47	0.53
	0.0081	0.0466	0.0111	0.0478	0.0243
PFTrDA	0.64	0.39	0.52	0.25	0.48
	0.0045	0.1088	0.0270	0.3209	0.0462
PFTeDA	0.68	0.56	0.62	0.47	0.57
	0.0018	0.0159	0.0059	0.0514	0.0145
PFBS	0.29	0.31	0.49	0.66	0.43
	0.2504	0.2149	0.0381	0.0034	0.0783
PFOS	0.93	0.78	0.82	0.83	0.77
	<0.0001	0.0001	<0.0001	<0.0001	0.0002

Supporting Data references:

American Public Health Association, American Water Works Association, and Water Environment Federation (APHA). 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed., Washington, D.C.

Miller WP, Miller DM. 1987. A micro-pipette method for soil mechanical analysis. *Commun. Soil Sci Plant Anal* 18:1-15.

Davies BE. 1974. Loss-on-ignition as an estimate of soil organic matter. *Soil Sci Soc Amer Proc* 38:150-151.

Henderson WM, Weber EJ, Duirk SE, Washington JW, Smith MA. 2007. Quantification of fluorotelomer-based chemicals in mammalian matrices by monitoring perfluoroalkyl chain fragments with GC/MS. *J Chromatogr B* 846:155-161.

Jenne EA, Crecelius EA. 1988. Determination of sorbed metals, amorphic Fe, oxidic Mn, and reactive particulate organic carbon in sediments and soils, In: Orio AA. (Ed), Proceedings of the 3rd Annual International Conference on Environmental Contamination. Selper Ltd., London, pp. 88-93.

Konwick BJ, Tomy GT, Ismail N, Peterson JT, Fauver RJ, Higginbotham D, Fisk AT. 2008. Concentrations and patterns of perfluoroalkyl acids in Georgia, USA surface waters near and distant to a major use source. *Environ Toxicol Chem* 27:2011-2018.

U.S. Environmental Protection Agency. Determination of trace elements in waters and wastes by inductively coupled plasma - mass spectrometry. EPA method 200.8 Revision 5.4, US EPA Cincinnati, OH 45268.

Washington JW, Ellington JJ, Jenkins TM, Evans JJ. 2007. Analysis of low concentrations of perfluorinated carboxylic acids in soils: issues with determination of presence & quantification at low levels. *J Chromatogr A* 1154:111-120.

Washington JW, Henderson WM, Ellington JJ, Jenkins TM, Evans JJ. 2008. Analysis of low concentrations of perfluorinated carboxylic acids in soils II: optimization of chromatography & extraction. *J Chromatogr A* 1181:21-32.